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Imaging whole-brain cytoarchitecture of mouse with MRI-based quantitative susceptibility mapping

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ABSTRACT

The proper microstructural arrangement of complex neural structures is essential for establishing the functional circuitry of the brain. We present an MRI method to resolve tissue microstructure and infer brain cytoarchitecture by mapping the magnetic susceptibility in the brain at high resolution. This is possible because of the heterogeneous magnetic susceptibility created by varying concentrations of lipids, proteins and irons from the cell membrane to cytoplasm. We demonstrate magnetic susceptibility maps at a nominal resolution of 10-μm isotropic, approaching the average cell size of a mouse brain. The maps reveal many detailed structures including the retina cell layers, olfactory sensory neurons, barrel cortex, cortical layers, axonal fibers in white and gray matter. Olfactory glomerulus density is calculated and structural connectivity is traced in the optic nerve, striatal neurons, and brainstem nerves. The method is robust and can be readily applied on MRI scanners at or above 7 T.

Introduction

The complex central nervous system (CNS) consists two main cell types, neurons and glia (Steinbusch, 1981). The average diameter of most neurons and glia cells is on the order of microns in the adult mouse brain (Geisert et al., 2002; Magavi et al., 2000). These cells are characterized by a wide variation in shape and are often location specific, e.g. pyramidal neurons are abundant in the cortical regions (Spruston, 2008). An understanding of these structures and their locations is essential to understand functional circuit properties and their relation to behaviors (Fields et al., 2015). However, mapping the entire brain at near cellular resolution is still challenging. Several imaging techniques are currently being used for acquiring high resolution data from a mouse brain, e.g. the conventional two-dimensional (2D) histology methods (Halliday et al., 2007; Lein et al., 2007) and optical microscopy (Dodt et al., 2007; Magavi et al., 2000). However, conventional 2D histology-based methods are limited by the orientation of available sections and sectioning-related damage and deformation. Optical approaches have limited tissue contrast for differentiating substructure within the brain. Dedicated high field (>7 T) animal MRI scanners have been shown to provide superior contrast and reveal fine anatomical details in the mouse brain (Jiang and Johnson, 2010; Wu et al., 2013). Advances in MRI techniques continue to improve resolution and contrast, providing a means to achieve a mesoscopic resolution (on the order of 10 μm) bridging gross neuroanatomy to the cellular architecture of the brain.

Several MRI contrast mechanisms that are thought to be sensitive to cellular organization have been applied to evaluate the mouse brain at high resolution. For example, current state-of-the-art DTI methods enable imaging an *ex vivo* adult mouse brain at approximately 40-μm isotropic resolution (Jiang and Johnson, 2010). But, DTI is inherently based on signal attenuation and is limited by T_2 and T_2^* decay, B_0 inhomogeneity, and limited signal-to-noise ratio (SNR) (Jaermann et al., 2006). Thus, DTI and other diffusion-based methods are problematic in resolving small fiber tracts, such as the structures of medium-sized spiny neurons (MSN) which are also complex with frequent branches (Matamales et al., 2009). Studies have shown that the use of MRI signal phase in gradient-echo (GRE) can uncover a fine structure in the brain tissue (Duyn et al., 2007; He and Yablonskiy, 2009; Rauscher et al., 2005). Phase imaging allows an enhanced contrast within gray matter and white matter that are not resolved with conventional imaging at ultra-high field (>7.0 T) MRI (Abduljalil et al., 2003; Duyn et al., 2007; Marques et al., 2009). However, phase values are non-local, i.e. the phase at one location not only depends on the local tissue properties but also depends on the neighboring magnetic susceptibility distribution.

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Quantitative susceptibility mapping (QSM) addresses this limitation by computing the spatial distribution of the underlying source of the phase, i.e. magnetic susceptibility (Bilgic et al., 2012; Carpenter et al., 2016; Li and van Zijl, 2014; Liu, 2010; Liu et al., 2014, 2015; Schweser et al., 2010; Wei et al., 2016; Xie et al., 2015). QSM reveals excellent image contrast and quantifies the magnetic properties of brain tissue, affected by e.g., iron in the cell body and myelin in the axons (Argyridis et al., 2014; Benner et al., 2013; Bilgic et al., 2012; Lee et al., 2012; Liu et al., 2012; Wang and Liu, 2015), which indicates that magnetic susceptibility may be used to improve the spatial resolution and contrast for cytoarchitecture of the whole brain. Current QSM techniques suffer from the severe streaking artifacts in the computed susceptibility maps from the single-orientation data (Li et al., 2015; Liu et al., 2013; Wei et al., 2015). A number of recent studies have aimed to improve the accuracy of susceptibility estimation and reduce the streaking artifacts (Li et al., 2015; Liu et al., 2013; Wei et al., 2015; Wu et al., 2012). One method called the streaking artifact reduction for quantitative susceptibility mapping (STAR-QSM) reduces streaks by limiting the strong susceptibility components (Wei et al., 2015).

In this study, mouse brains (n = 2) were scanned *ex vivo* at a nominal 10-μm isotropic resolution using a three-dimensional (3D) GRE sequence at 9.4 T. We applied STAR-QSM to address current issues of streaking artifacts. In this dataset, QSM offers a powerful tool to resolve fine detailed magnetic susceptibility contrast in many structures, e.g. retina cell layers, olfactory sensory neurons, corpus callosum, putamen axon, cerebral cortical layers, barrel cortex, hippocampus layers, cerebellum, striatal neurons, and the brainstem. Using STAR-QSM, we are able to achieve in susceptibility mapping at a resolution and contrast exceeding traditional MR images.

Materials and methods

Perfusion and fixation

Mice (adult male C57BL/6) were provided with free access to food and water before experiments. Mice were anesthetized with isoflurane, a midline abdominal incision was made, and a catheter was inserted into the heart. Transcardial perfusion fixation was used with inflow to the left ventricle and outflow from the right atrium. The animals were perfused with saline and 0.1% heparin followed by a solution of 2.5 mM ProHance (Gadoteridol, Bracco Diagnostics Inc., Princeton, NJ) in 10% formalin. Both saline and ProHance-formalin were perfused at 8 ml/min for 5 min using a perfusion pump. Brain specimens were immersed in ProHance-formalin overnight and then immersed in solution of 2.5 mM ProHance in 10 mM phosphate buffered saline the next day. Imaging was obtained several weeks later. The study was approved by the local Institutional Animal Care & Use Committee.

Data acquisition

The specimen was firmly affixed in an 11-mm polyethylene tube filled with Fomblin (fluoropolyether; Ausimont, Inc., Morristown, NJ) to mitigate tissue dehydration and reduce large susceptibility distortions at the specimen surface. MR experiments were performed using a 9.4 T (400 MHz) 8.9-cm vertical bore Oxford magnet with shielded gradients of 2200 mT/m. The specimen was scanned with the long axis (rostral–caudal axis) of the mouse brain oriented perpendicular to the main magnetic field direction. A 3D spoiled multiecho GRE sequence was used with the following parameters: field of view (FOV) = 22 × 11 × 10 mm^3, matrix size = 2200 × 1100 × 1000 resulting in a nominal voxel size of 10 × 10 × 10 μm^3, TE_1/TE_2 = 6.8/16.4 ms, TR = 35 ms, and flip angle = 90°. Acquisition time for each scan was 10 h 42 min. Nine or ten signal averages were acquired to achieve adequate SNR making the total scan time around 100 h.

Data reconstruction

The images were reconstructed with 3D Fast Fourier transform using the complex k-space data and then separated into magnitude and phase. The magnitude images from individual scans (Fig. 1A) were averaged to achieve a higher SNR. The averaged magnitude image was used for the extraction of the brain tissue mask. Meanwhile, the raw phase from each scan was unwrapped separately using the Laplacian-based phase unwrapping (Li et al., 2011), the phase images at two echoes from a single scan were averaged. The phase images from all these individual scans (Fig. 1E) were then averaged prior to QSM (Fig. 1F), and the local tissue phase (Fig. 1G) is obtained by removing the background phase via spherical mean value filtering (SMV) with a variable diameter towards the brain boundary (Wu et al., 2012). The variable radius of the SMV filter increased from 1 pixel at the brain boundary to 25 towards the center of the brain. The filtered phase image was further denoised by a block matching grouping approach based on a sparse representation in transformed domain (Maggioni et al., 2013). Briefly, the sparsity is achieved by grouping similar 3D image cubes into the 4D "group". Four one-dimensional linear transform operators were separately applied to each dimension of the group. The obtained four-dimensional group spectrum is then coefficient shrunk by a thresholding operator. The estimate of the group with reduced noise is finally produced by applying the inverse four-dimensional transform to the shrunk spectrum. We set the size of the cubes to 5 × 5 × 5 for grouping. The separable four-dimensional transform is a composition of a 3-D Haar transform in the cube dimensions and a 1-D Haar wavelet in the grouping dimension as the default setting. More details about the algorithm procedure can be found in the Supplementary material and in the reference (Maggioni et al., 2013). Lastly, the denoised tissue phase map (Fig. 1H) is processed using the STAR-QSM algorithm (Wei et al., 2015) to obtain the susceptibility maps (Fig. 1I).

Image analysis and visualization

A 3D Hough transform was used to segment the glomeruli from the olfactory bulbs. The technique for this study was based on a 2D circular Hough transform implemented by Peng et al. (2007) which was extended into 3D to search for spheres in volume data (Xie et al., 2012). Glomeruli were assumed to be spherical at the current resolution. The algorithm was performed in MATLAB (MathWorks Inc., Natick, MA) and is made available on MATLAB Central File Exchange (www.mathworks.com/matlabcentral/fileexchange/48219).

Visualization and volume rendering were accomplished using a combination of ImageJ (http://rsbweb.nih.gov/ij/), Avizo (Visualization Sciences Group, Burlington, MA), and Vitrea (Vital Images, Inc., Minnetonka, MN). Specifically, the barrel cortex (neuron somata) was visualized using Vitrea. Here, a curved surface was manually selected in the vibrissal cortex and then flattened to visualize the barrels. Volume rendering of the olfactory glomeruli and optic nerves was performed using Avizo. The striatum area was first manually segmented as outlined in red in Fig. 5B. Then striatal tracts were followed using a seeded region growing on the QSM images. Skeletonization and surface renders were then performed and visualized using Avizo.

Results

High contrast in the brain tissue can be observed in both magnitude and susceptibility images (Fig. 2A & B). Note that susceptibility values are inverted and the bright glomeruli are diamagnetic. Higher structural contrast can be observed in susceptibility images (Fig. 2B). For instance, the glomeruli in the olfactory bulb, the mitral cell layer, and the hippocampal cell layers can be seen in much higher detail in QSM compared to magnitude. Furthermore, the olfactory bulb, putamen, and cerebellum